







## Research Article

# Habitat and diet interactions in a lowland temperate river suggests no direct impact of non-native monkey goby (*Neogobius fluviatilis*) on native spined loach (*Cobitis taenia*)

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## Abstract

Non-native species can negatively impact native ecosystems, but their introduction does not always lead to major ecological changes. This study examines interactions between the non-native monkey goby (*Neogobius fluviatilis*) and the native spined loach (*Cobitis taenia*) through field observations and controlled laboratory experiments. We assessed microhabitat use, dietary similarities, and behavioral interactions between the two species. Field results revealed overlapping microhabitats dominated by coarse sand, but with no significant displacement of the spined loach by the monkey goby. Laboratory trials further supported these findings, showing no competitive interference in habitat selection, with both species co-occupying preferred substrates (coarse sand). Diet analysis indicated limited trophic overlap (17%), suggesting resource partitioning rather than direct competition. The results show no evidence that the non-native monkey goby exerts direct harmful effects on the native spined loach in the river studied. Instead, our study highlights the potential for coexistence, emphasizing the need for nuanced approaches in assessing the ecological impacts of non-native species. However, it should also be noted that our results are time and space-limited, and indirect and/or long-term effects, not captured by this study, may exist. Current research contributes to a broader understanding of complex biotic interactions between non-native and resident species.

**Key words:** Competition experiment, diet analysis, stable isotopes, substrate preferences



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## Introduction

Increasing anthropogenic pressure significantly affects aquatic ecosystems, leading to homogenization through habitat modifications and species invasions (Marr et al. 2013). In response, extensive efforts have been made globally and nationally, including the implementation of regulations, risk assessments of non-native species, and management strategies aimed at eradicating or controlling their populations (Britton 2023). Despite these efforts and advancements in understanding the biology of non-native species, their proliferation persists, highlighting the urgent need for studies confirming their impact to inform and implement effective control measures.



The most successful invaders of European inland waters include Ponto-Caspian gobies, with the round goby *Neogobius melanostomus*, serving as a prominent model species due to its significant negative impacts (Cerwenka et al. 2023). Conversely, other goby species such as the monkey goby *Neogobius fluviatilis*, racer goby *Babka gymnotrachelus*, bighead goby *Neogobius kessleri*, and western tubenose goby *Proterorhinus semilunaris* have been relatively understudied despite actively expanding their ranges (Grabowska et al. 2023). In Europe, the primary pathways for the expansion of Ponto-Caspian gobies are the central and southern corridors (Bij de Vaate et al. 2002; Semenchenko et al. 2011). Over the past three decades, the monkey goby, racer goby, bighead goby, and western tubenose goby have utilized those corridors to disperse. While the round goby typically remains in main rivers, the other gobies successfully expanded into tributaries.

The ecological impact of the monkey goby remains poorly understood. Experimental laboratory studies have not shown any detrimental effects on native species, such as the European bullhead *Cottus gobio* (Błońska et al. 2016), river bullhead *Cottus perifretum* (Van Kessel et al. 2011), stone loach *Barbatula barbatula* (Van Kessel et al. 2011). Irrespective of the season (spawning / non-spawning) the monkey goby influence on the native counterpart was similar to that of conspecifics (Błońska et al. 2016). Other studies showed the advantages of the monkey goby over its native analogue species consisting of lower energy expenditures on metabolism under increased temperature (Kłosiński et al. 2024) and enhanced boldness and exploratory behavior, allowing it to expand across the open riverbed (Augustyniak et al. 2024).

Given that the monkey goby is constantly and successfully increasing its non-native range, establishing abundant populations (e.g. in Poland it covered 340 km in five years; Bylak and Kukuła 2024) it is unlikely to assume that its presence is indifferent to native species, in particular those of similar requirements. Apart from the already existing intraspecific competition, the arrival of non-native gobies can add to the interspecific competition, though it would vary with season and fish species (Błońska et al. 2016). All the above mentioned research on interactions between native fish species and the monkey goby was performed under controlled laboratory conditions (Van Kessel et al. 2011; Błońska et al. 2016; Kłosiński et al. 2024), necessitating a more comprehensive approach that includes both field and laboratory observations to present not only potential, but also direct influences of the invasive species. Such an approach reveals that instead of competition suggested based on lab experiments, an alternative scenario is quite often implemented in ecosystems after arrival of an invasive species, i.e. resource and habitat partitioning to avoid competition with ecologically similar native species (Tran et al. 2015; Kakareko et al. 2016; Britton et al. 2018). However, the impact of non-native species is known to be context- and site-dependent. The same species might pose a serious threat in one location while having a mild effect in another (Błońska et al. 2024).

One of the native fish species that can be potentially affected by the expansion of the monkey goby invasion is the spined loach *Cobitis taenia* (Błońska et al. 2024). Both species inhabit a wide range of freshwaters, including rivers, lakes and reservoirs (Robotham 1978; Grabowska et al. 2023). They have similar habitat preferences, e.g. for sandy bottom (Robotham 1978; Copp and Vilizzi 2004; Pietraszewski 2015; Płachocki et al. 2020) and a habit of burying themselves in the substrate, possibly as an antipredator strategy (Kakareko 2011 in case of gobies). Besides, both feed on benthic macroinvertebrates, mainly chironomid larvae (Marszał et al. 2003; Grabowska et al. 2009; Jazdzewski 2020; Didenko et al. 2021). Faunistic fish studies



showed that the spined loach co-occurred with the monkey goby at each of 22 sites sampled along the distance of almost 360 km in the Eastern Bug River (Penczak et al. 2010). Their generally similar lifestyle and requirements suggest that they should have equally similar patterns of in-stream distribution and abundance that can potentially result in competitive interactions. The spined loach occurs across almost the whole of Europe and central Asia (Bohlen and Ráb 2001; Janko et al. 2007), but is endangered in many European countries (Kotusz 1996). The species is listed in Appendix III of the Bern Convention and Annex II of the EC Habitats Directive (92/43/EEC) on the Conservation of Natural Habitats and of Wild Fauna and Flora. The rapid spread of the monkey goby in the Pilica River has coincided with the spined loach decrease (own unpublished data). On the other hand, the observed decline of spined loach abundance and occurrence might be caused by the synergic effect of many other factors that alter environmental conditions in a river negatively for spined loach, and the arrival of the non-native species may only contribute to the decline or be not harmful at all. The causal relationship between these phenomena is unknown, as the interactions between these two species have never been investigated.

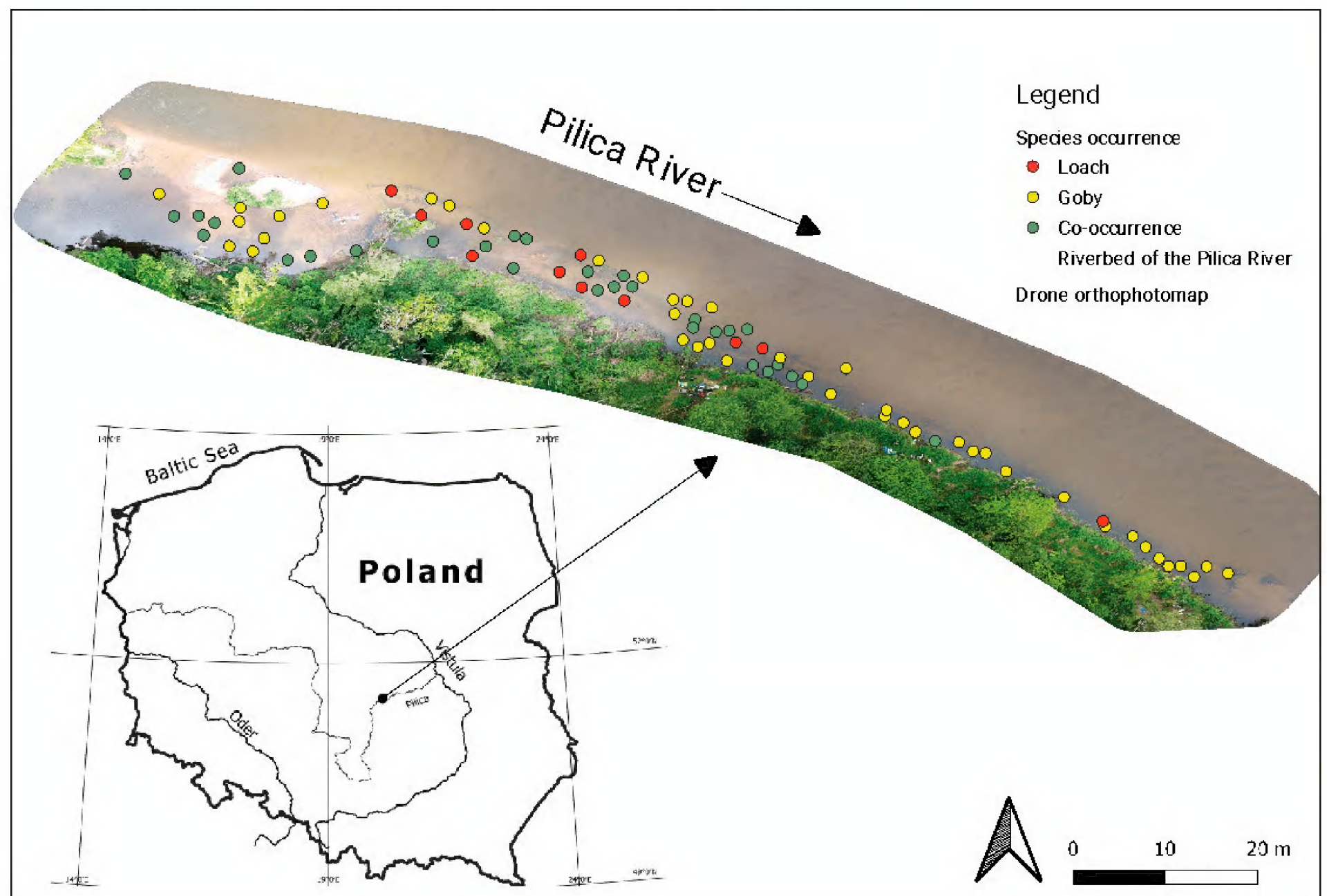
Therefore, we conducted an extensive study on the interactions between the monkey goby and spined loach to fill this knowledge gap and verify the impact of the monkey goby. Our study combines field observations of microhabitat occupation and diet overlap at sites of co-occurrence, utilizing traditional stomach content analysis and contemporary stable isotope analysis, along with laboratory experiments on habitat preferences and competition for limited resources. We hypothesised that (i) the monkey goby will competitively displace the native spined loach through interference competition and that (ii) the monkey goby and spined loach have overlapping dietary niches, indicating potential competition for food resources. We tested the first hypothesis in the laboratory through direct assessment of competition for limited habitat resources and in a field study, investigating the co-occurrence of both species in the same microhabitats. The second hypothesis was tested with stable isotope analysis and stomach content analysis.

## Materials and methods

### Field research

Field campaign to collect data on species occurrence was conducted in May 2024. May was chosen to collect samples to avoid peaks of macroinvertebrate density in early spring (before the emergence of diapaused generations) and in summer (after emergence and growing new generations) (Murphy and Giller 2000). Specimens of monkey goby and spined loach were sampled from the Pilica River near Spała village, Poland (Fig. 1), by electrofishing (EFGI 650; BSE Specialelektronik Bretschneider, Germany) using point sampling along the river stretch of  $100 \times 4$  m (length and width, respectively; 95 points in total). Electrofishing, even with a low-power backpack electrofisher as used in our study, could potentially lead to localized avoidance behavior in fish, where individuals escape the immediate area of the anode and move to nearby unsampled locations. This may result in a slight underrepresentation of certain individuals at sampling points or influence their spatial distribution. However, we believe that the potential effects of this bias are mitigated for the following reasons: (1) wading upstream ensures that only fish in close proximity to the anode are affected; and (2) the specific behavior of focal





**Figure 1.** Map of the sampling site located on the Pilica River, where monkey goby (*Neogobius fluviatilis*) and spined loach (*Cobitis taenia*) were collected in May 2024.

species, which burrow in sediment and require time to dig out to escape while electrocuted. Collected fish were placed in buckets and the point of collection was marked. At the bank, individuals were counted and measured to the nearest 1 mm. The whole sampling area with marked points was photographed using a drone (Fig. 1). At each point, a sample of substrate was also taken using a tubular sampler with a cross-sectional area of 25 cm<sup>2</sup>, which was inserted into the sediment to a depth of 15 cm. All collected substrate samples were dried in the lab and sifted (mechanical sieve shaker LAB-11-200) providing data on the percentage of particular substrate fractions (from 128 to 0.063 mm) in individual microhabitats of each fish. In total, 40 specimens of both species (20 monkey goby and 20 spined loach) were taken to compare diet and trophic ecology. All fish were euthanized using an overdose of clove oil.

### Diet composition

All fish collected for the gut content analysis were preserved in 4% formaldehyde solution. They were then measured (total length, TL) to the nearest 1 mm and weighed (W) to the nearest 10 mg (monkey goby: 77 ± 22 mm; spined loach: 79 ± 14 mm, on average ± SD). Gut contents were weighed to the nearest 1 mg and stored in glycerin. Food items were subsequently identified to the lowest possible



level of taxonomy; i.e. to order, family or species and/or genus where possible, under a stereomicroscope (Nikon SMZ1000, Japan) and counted. The total number and weight of each prey type were estimated for each fish. The analysis of the diet was based on the percentage of biomass of each prey ( $\%W_i$ ). Prey items were combined by taxon and quantified by the frequency of occurrence ( $\%FO_i$ ) and percentage of biomass ( $\%W_i$ ) (Hyslop 1980). For each food category, the index of importance (IRI) was calculated (Pinkas 1971) and its standardized value ( $\%IRI$ ) (Cortés 1997) was estimated as:

$$IRI_i = \%FO_i \times \%W_i$$

$$\%IRI_i = 100 \text{ } IRI_i / \Sigma IRI_i$$

where  $IRI_i$  is the IRI value for the  $i^{\text{th}}$  prey category and  $\Sigma IRI_i$  is the total IRI for all prey categories.

To estimate diet overlap, the Schoener  $\alpha$  index was used. This index was calculated as:

$$\alpha = 1 - 0.5 \Sigma |p_{ix} - p_{iy}|$$

where  $p_{ix}$  and  $p_{iy}$  are the biomass proportions of the  $i^{\text{th}}$  food resource used by monkey goby and spined loach. The Schoener  $\alpha$  is the most commonly used niche overlap measure. Values of the index lie between 0, indicating no overlap, and 1, when diets are identical, whereby overlap values exceeding 0.6 are regarded as high or biologically significant (Wallace 1981). For all indices, average values and their standard errors were obtained using the jackknife technique (Krebs 1999).

### Stable isotope analysis

Ten specimens of each species were used for stable isotope (SI) analysis (mean TL  $92 \pm 26$  mm and  $81 \pm 10$  mm for monkey goby and spined loach, respectively). Specimens for SI analysis were preserved in ice and stored at  $-20^\circ\text{C}$  before defrosting. A sample of dorsal muscle tissue was excised from each individual for bulk carbon and nitrogen stable isotope analysis (SIA). White muscle tissue, which has a lower variability in nitrogen isotopic signature compared to other tissues, does not require acidification to remove inorganic carbonates (Pinnegar and Polunin 1999). The sampled muscles were then dried in an oven at a constant temperature of  $60^\circ\text{C}$  for 24 hours before being ground to a fine powder using an agate pestle and mortar. Samples of  $1 (\pm 0.1)$  mg of homogenized tissues were subsequently analyzed using a Thermo Finnigan Delta Plus Advantagean isotope ratio mass spectrometer at the Biological and Chemical Research Centre in Warsaw, Poland. The isotope compositions were expressed in  $\delta$  notation (‰), calculated as  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$ , where  $R$  represents the  $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$  ratios. The standards used were Vienna Pee Dee Belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen. To ensure the reliability of the isotopic analysis, samples were analyzed in duplicates. The average standard errors were 0.03‰ for  $\delta^{13}\text{C}$  and 0.11‰ for  $\delta^{15}\text{N}$ .



## Laboratory experiment

In autumn 2022, monkey goby and spined loach individuals were collected from the same site as used for the field sampling. The fish were transported to the laboratory in aerated containers. They were then placed in 70-liter aerated aquaria with a water temperature maintained at 17–18 °C. The aquaria were connected to a water circulation system. Each aquarium was equipped with shelters made from 5 cm long PVC pipe halves and artificial plants. The bottom was covered with a thin layer of sand. The number of fish per aquarium ranged from 5 to 8, with more shelters than fish to prevent competition outside the experimental arena. The fish were grouped by species and then by size to ease further matching of individuals for experiment (mean TL 102 mm and 92 mm for the monkey goby and spined loach, respectively). Every other day, they were fed frozen chironomid larvae. The photoperiod was set to 10 hr of light and 14 hr of darkness to mimic natural light conditions during that time. Both stocking aquaria and the experimental tanks were located in the same laboratory room.

Plastic containers (IKEA, Samla) with a capacity of 15 liters (39 × 28 × 14 cm) were used to conduct the experiments. The containers were lined with black plastic wrap to limit the access of stimuli to the experimental arena (also preventing visibility of individuals in adjacent tanks). The containers were divided in half. Approximately 3–4 cm layer of substrate was added at the bottom. Above each container, a camera was installed to record fish behavior (Gemini Technology, GT-CH21C5-28VFW). The water was aerated before starting the experiment and changed before each trial (aerating stones were removed during the experiment).

The experiments were performed in January/February 2023. At first (Experiment 1), we assessed species preferences towards three substrates: fine sand (grain diameter 0.125–0.250 mm), coarse sand (0.5–1 mm) and granule (2–4 mm). Single fish were exposed to two types of substrates (three different treatments: fine sand vs. coarse sand, coarse sand vs. granule). Fish were observed for 20 h and time spent buried in the particular substrate or on exploration (swimming in the tank / not buried) was measured. Those observations enabled us to designate substrate preferred by the majority of individuals (i.e. coarse sand, see the results) as well as avoided one (granule), which were then used in the competition experiment (see below). Each treatment was replicated 10 times.

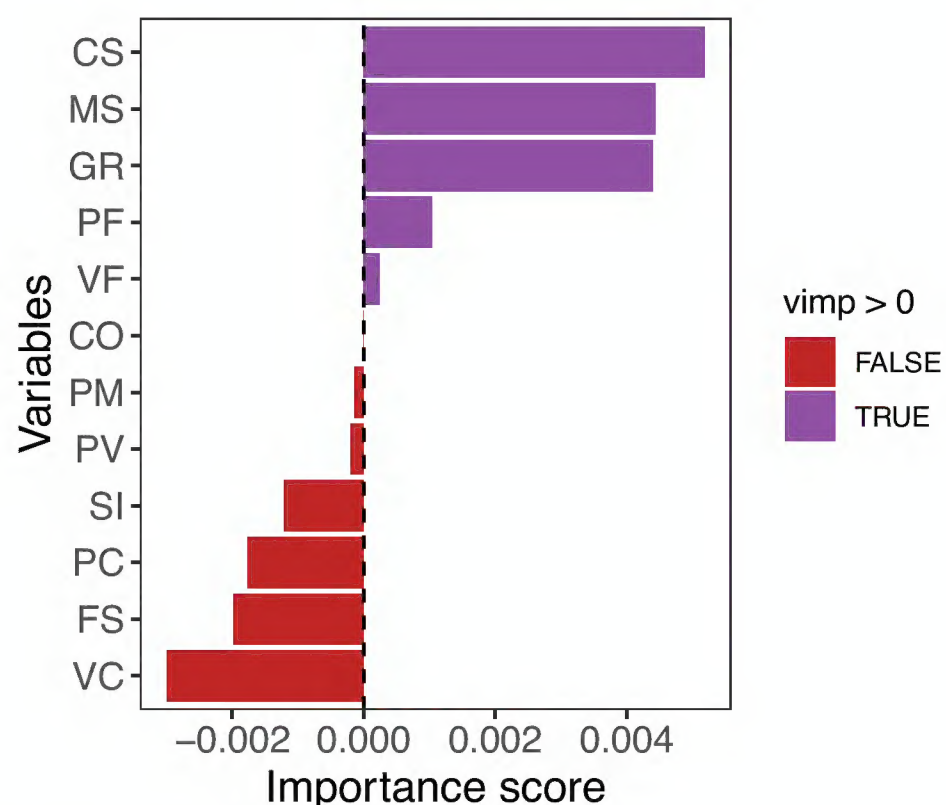
To evaluate interactions between the monkey goby and spined loach, both species were subject to the following experimental protocol (Experiment 2). The experimental arena was the same as in Experiment 1, with one half filled with coarse sand (preferred substrate, hereafter referred to as “sand”) and the other with granule (avoided, see the results of Experiment 1). A single fish was placed into an experimental tank and given 24 h for acclimation. Then, an intruding fish was introduced (making the first individual a resident) and both individuals were recorded for 20 h, to note their behavior (buried / exploring / aggressive). We tested all species combinations (spined loach vs. spined loach; spined loach vs. monkey goby; monkey goby vs. monkey goby; monkey goby vs. spined loach; resident vs. intruder, respectively), each replicated 10 times. Specimens were used only once in the competition experiment, however, as the spined loach is partially protected by law in Poland, we had to re-use individuals from the preference experiment. All actions were approved by the Local Ethic Committee (52/ŁB251/2022) and the General Directorate of Environmental Protection (WPN.672.8.2022.AGr and WPN.6401.136.2024.BWo).



## Data analysis

Previous studies, including Kakareko et al. (2016), have shown that the impact of non-native gobies, such as the racer goby, varies with fish size, with smaller individuals experiencing more adverse effects. Based on this, and to avoid potential biases from pooling all individuals into a single group, we divided both monkey gobies and spined loaches into two groups: small (below 70 mm) and large (70 mm or above). This categorization was informed by the size distribution of fish collected at the site and supported by available literature (e.g. Kakareko 2011; Płachocki et al. 2020).

To identify the most important drivers of habitat use of monkey goby and stone loach in the wild, we used a random forest model with the "rfsrc" function from the randomForestSRC R package. Random forest was selected due to its effectiveness in handling multiple predictor variables and complex interactions without requiring strict parametric assumptions (Breiman 2001), making it well-suited for identifying key drivers in habitat use studies (Kurtul et al. 2024). The dependent variable in this analysis was the abundance of each species, as measured at each sampling point. Individual sampling points served as replicates in the model, allowing us to capture the variation in habitat use across locations. Variable importance in the random forest (RF) was determined by measuring the decrease in prediction accuracy when each variable was randomly permuted, keeping all other variables constant (i.e. a greater decrease in accuracy indicates a more important variable). We used a total of 2,000 decision trees and five nodes in each tree. The out-of-bag (OOB) error rate, which measures the model's predictive accuracy, was 0.12, with an out-of-bag fit value 0.1, indicating a reasonably robust model. To interpret the influence of each predictor on habitat use, we considered the variables mentioned in Fig. 2 and evaluated their impact on species abundance.



**Figure 2.** Relative variable importance of habitat type assessed by the applied random forest for affecting the presence of monkey goby (*Neogobius fluviatilis*) and spined loach (*Cobitis taenia*). The variables considered are as follows (particle size ranges in mm): CS (coarse sand, 1–0.5), MS (medium sand, 0.5–0.25), GR (granule gravel, 4–2), PF (fine pebble, 16–8), VF (very fine sand, 0.125–0.063), CO (cobble, 128–64), PM (medium pebble, 32–16), PV (very fine pebble, 8–4), SI (silt, < 0.063), PC (coarse pebble, 64–32), FS (fine sand, 0.25–0.125), and VC (very coarse sand, 2–1). Purple indicates positive effects, while red indicates negative effects. VIMP refers to Variable Importance.



Following on the results of the random forest analysis, which identified the primary substratum (i.e. coarse sand) as the most important predictor (see the results), we applied a Zero-Inflated Negative Binomial (ZINB) model to assess potential interspecific interactions between monkey goby and spined loach using the “glmmTMB” function from the glmmTMB R package (Brooks et al. 2017). In each model, the response variable was the count of either small or large individuals from both goby and loach species. Fixed effects included the counts of the other fish groups (small goby, large goby, small loach, large loach) and the presence of coarse sand (selected based on the random forest analysis results) to assess potential competitive interactions and differences in habitat preferences among species. The ZINB model was chosen due to the high frequency of zero counts in the data and the over-dispersion of fish counts. This model allowed us to explore how the abundance of small and large individuals of each species were influenced by coarse sand as a habitat feature. Before fitting the model, we conducted a thorough data exploration following the guidelines of Ieno and Zuur (2015). This process includes checking for missing values, identifying outliers in both response and explanatory variables, assessing homogeneity and zero inflation in the response variable, evaluating collinearity among explanatory variables, ensuring balance within categorical variables, and examining the relationships between the response and explanatory variables. All candidate models were validated using the DHARMa package in R (Hartig and Lohse 2022). The “simulateResiduals” function was used to simulate standardized residuals, allowing us to check key model assumptions by examining residual patterns for potential deviations (such as homogeneity of variance, normality, and outliers). Additionally, the “plotQQunif” function generated a Q-Q plot to further assess residual normality. Tests for model dispersion and outliers were also performed during this validation step.

To analyze the differences in the diet between fish species, one-way permutation analysis of similarity (ANOSIM, Bray-Curtis similarity index) was used. The significance level of the R statistics was calculated using 9999 permutations of the dataset. Then, similarity percentage procedure (SIMPER) was applied to distinguish which prey taxa had the greatest contribution to the dissimilarity of the diet of investigated fish species. All multivariate techniques for analyzing diet data were conducted using the PAST v3.15 software (Hammer et al. 2001).

To assess the overlap between the isotopic niches of the two species, we used three complementary approaches. First, we identified if the occupied trophic niches were significantly different using a permutational univariate analysis of variance (PERANOVA) on the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the two species, with Euclidean distance and 9,999 permutations using the “adonis2” function implemented in the R package vegan (Oksanen 2012). Then, the ratio between the overlap area and the sum of both niche areas for the 95% (maximum likelihood and Bayesian ellipses- $\text{SEA}_B$ ) and corrected standard ellipse areas for the 40% ( $\text{SEA}_C$ ) standard ellipse areas (SEA) were calculated using the R package SIBER (Jackson et al. 2011). Finally, the directional probability of an individual of a species to occur within the niche of the other species (considered as the 95% and 40% standard ellipse area) was estimated applying a Monte Carlo estimation (chain-length: 10,000 steps) using the R package nicheROVER (Swanson et al. 2015).

To check substratum selectivity in Experiment 1 and 2, we compared percentages of time spent by the fish in coarse sand to a theoretical value of 50% (assuming no selectivity) using one sample Wilcoxon tests. We used a General Linear Model



(GLM) to test the effect of (1) fish species, (2) substratum configuration (coarse & fine sand vs coarse sand & gravel) and (3) time (day vs night, within-subject factor) on percentage of time spent by single fish in movement (proxy for fish activity) in Experiment 1. We distinguished between day and night to account for the temporal factor, acknowledging that both species exhibit variable diel activity patterns (Grabowska et al. 2009; Błońska et al. 2016; Jażdżewski 2020).

We tested factors affecting fish behavior in Experiment 2: (1) percentage of time spent in sand and (2) percentage of time spent in movement using a set of GLMs, separately for each species. First, we tested the effect of (1) intruder presence and (2) intruder species using the measurements of single fish later becoming residents exposed to the presence of intruders. This analysis allowed us to check if the fish respond to the introduction of an intruder and whether this response depends on the intruder species. Second, we compared the behavior of intruders depending on (1) resident species to which they were exposed. This analysis allowed us to check if intruders behaved differently depending on the species of the resident individual. Third, we compared the behavior of fish within single-species pairs depending on (1) individual status (resident vs intruder). This analysis allowed us to check if intruder individuals behaved differently than resident fish staying in the arena for a longer time. In the above models, intruder presence and individual status were modelled as within-subject factors, as measurements were taken twice for the same individual (without and with the intruder), or for two individuals exposed together (resident and intruder), respectively. These models additionally included time (day vs night, within-subject factor) and relative size (resident/intruder length ratio) to control for their effects.

Initial models included all main effects and their interactions. Then, non-significant higher order interactions were dropped from the model in a simplification procedure. As needed, sequential Bonferroni corrected Fisher LSD tests were used as a post-hoc procedure to disentangle significant effects in the models.

## Results

### Field research

The random forest identified that sand (coarse and medium), granule and fine pebble were the most important variables in predicting presence of monkey goby and spined loach (Fig. 2).

Based on the results of random forest, coarse sand was the primary habitat (Fig. 2). The GLM models indicate that the tested substrate type, as well as conspecifics or non-native goby of different sizes did not have a significant effect on the counts of large and small loach (Table 1), suggesting that other factors may be influencing their habitat use. The marginally non-significant effects of the substrate type on large loach and for small goby on small loach count warrant further investigation (Table 1C, D, respectively).

### Diet composition

The analysis of alimentary tract contents showed that, among 19 food categories, the monkey goby fed primarily on Ephemeroptera, Chironomidae, Trichoptera, and *Asellus aquaticus*, complemented by Bivalvia. In turn, the spined loach exploited mainly Chironomidae, Trichoptera, and Simuliidae. Their gut also contained



sand, which was probably consumed additionally with other prey types (Suppl. material 1: table S1). The remaining food categories identified in the diet can be considered as rarely chosen on the basis of their amount and frequency in the diet (Suppl. material 1: table S1). IRI values also indicated that the most important prey for monkey goby were Ephemeroptera, Chironomidae, Trichoptera and *Asellus aquaticus* (48.2%, 21.9%, 12.4%, 12.1% IRI, respectively), what constituted 94.6% IRI in total. In the case of spined loach, Chironomidae, Trichoptera achieved the highest IRI values (46.9%, 25.2%, respectively). The remaining prey categories had lower, equal shares in the fish diet (Suppl. material 1: table S1).

The diet composition and importance of food items differed markedly between fish species (ANOSIM: R-statistic = 0.276,  $p < 0.0001$ ). SIMPER analysis showed that dissimilarity in the diet composition of fish sampled was based on Ephemeroptera, Chironomidae, Insecta remains, *Asellus aquaticus*, Trichoptera and Bivalvia (Table 2). These five categories together constituted over 77.4% of cumulative dissimilarity in the diet between fish species.

Both fish species consumed a wide spectrum of prey groups, but the Schoener  $\alpha$  index ( $0.49 \pm 0.039$ ) showed no distinct diet overlap.

**Table 1.** GLMM analysis of the effect of substratum type and co-occurring fish on the counts of the studied fish species and size classes.

| Response variable    | Fixed factors    | Log-mean | 95% CI     | P     |
|----------------------|------------------|----------|------------|-------|
| A. Large goby count  | Intercept        | -0.78    | -1.92–0.37 | 0.184 |
|                      | Substrate (sand) | 0.01     | -0.01–0.03 | 0.511 |
|                      | Small goby       | -0.07    | -0.36–0.23 | 0.657 |
|                      | Large loach      | 0.03     | -0.26–0.33 | 0.823 |
|                      | Small loach      | -0.05    | -0.50–0.40 | 0.826 |
| B. Small goby count  | Intercept        | 0.55     | -0.19–1.29 | 0.146 |
|                      | Substrate (sand) | -0.01    | -0.02–0.01 | 0.225 |
|                      | Large loach      | -0.19    | -0.42–0.05 | 0.125 |
|                      | Small loach      | 0.18     | -0.09–0.45 | 0.180 |
|                      | Large goby       | -0.04    | -0.29–0.21 | 0.753 |
| C. Large loach count | Intercept        | 0.63     | -0.57–1.82 | 0.304 |
|                      | Substrate (sand) | -0.02    | -0.05–0.00 | 0.068 |
|                      | Small goby       | -0.25    | -0.57–0.07 | 0.124 |
|                      | Small loach      | 0.27     | -0.09–0.63 | 0.142 |
|                      | Large goby       | 0.11     | -0.22–0.43 | 0.513 |
| D. Small loach count | Intercept        | -1.37    | -3.05–0.31 | 0.110 |
|                      | Substrate (sand) | -0.01    | -0.04–0.02 | 0.628 |
|                      | Small goby       | 0.30     | -0.02–0.62 | 0.065 |
|                      | Large loach      | 0.29     | -0.08–0.67 | 0.127 |
|                      | Large goby       | -0.12    | -0.64–0.41 | 0.670 |

**Table 2.** Taxa contributing considerably (>5%) to the dissimilarity in diet between the monkey goby (*Neogobius fluviatilis*) and spined loach (*Cobitis taenia*) obtained from SIMPER analysis.

| Food category            | Dissimilarity |                |              |
|--------------------------|---------------|----------------|--------------|
|                          | Average       | Contribution % | Cumulative % |
| Ephemeroptera            | 21.73         | 25.30          | 25.30        |
| Chironomidae             | 16.33         | 19.01          | 44.31        |
| Insecta remains          | 8.72          | 10.16          | 54.47        |
| <i>Asellus aquaticus</i> | 7.94          | 9.24           | 63.70        |
| Trichoptera              | 6.69          | 7.79           | 71.49        |
| Bivalvia                 | 5.10          | 5.93           | 77.43        |



Stable isotope analysis

The isotopic niches of the monkey goby and loach were statistically differentiated (pseudo $F_{1,19} = 23.97$ ,  $P < 0.001$  for  $\delta^{13}\text{C}$  and pseudo $F_{1,19} = 6.09$ ,  $P < 0.02$  for  $\delta^{15}\text{N}$ ), indicating that there was no strong competition between the species. In terms of the 95% Bayesian standard ellipse area ( $\text{SEA}_B$ ), the overlap of the monkey goby with loach was 17.2%. Monkey goby exhibited a wider  $\text{SEA}_B$  (Fig. 3) and isotopic metrics (Suppl. material 1: table S2) compared to loach. Considering  $\text{SEA}_B$ , the potential directional overlap of monkey goby with loach was 35.9%, whereas it was 28.9% when considering loach overlapping monkey goby. When considering the 40% corrected standard ellipse areas ( $\text{SEA}_C$ ), the overlap potential was much lower. Monkey goby had a small overlap with loach (5.2%). The probability of loach overlapping monkey goby was 2.4%.

Laboratory experiment

In Experiment 1, single individuals of the spined loach selected coarse sand over fine sand and granule (Fig. 4A, Table 3). The monkey goby preferred sand over granule but did not discriminate between two types of sand. The spined loach were more active than monkey goby (Suppl. material 1: fig. S1A). Moreover, the goby were more active at night than in daylight, whereas the same tendency for the loach was non-significant, resulting in a significant species\*time interaction (Suppl. material 1: table S3).

In Experiment 2, the spined loach always occupied an exclusively sandy substratum, irrespective of their status (single, resident, intruder), the presence and species of the accompanying individual and time of the day (Fig. 4B, Suppl. material 1: table S4). In most cases, the monkey goby also exhibited such preference, except intruder gobies in single-species configurations and intruder gobies facing the spined loach in daylight (Fig. 4B, Suppl. material 1: table S4).

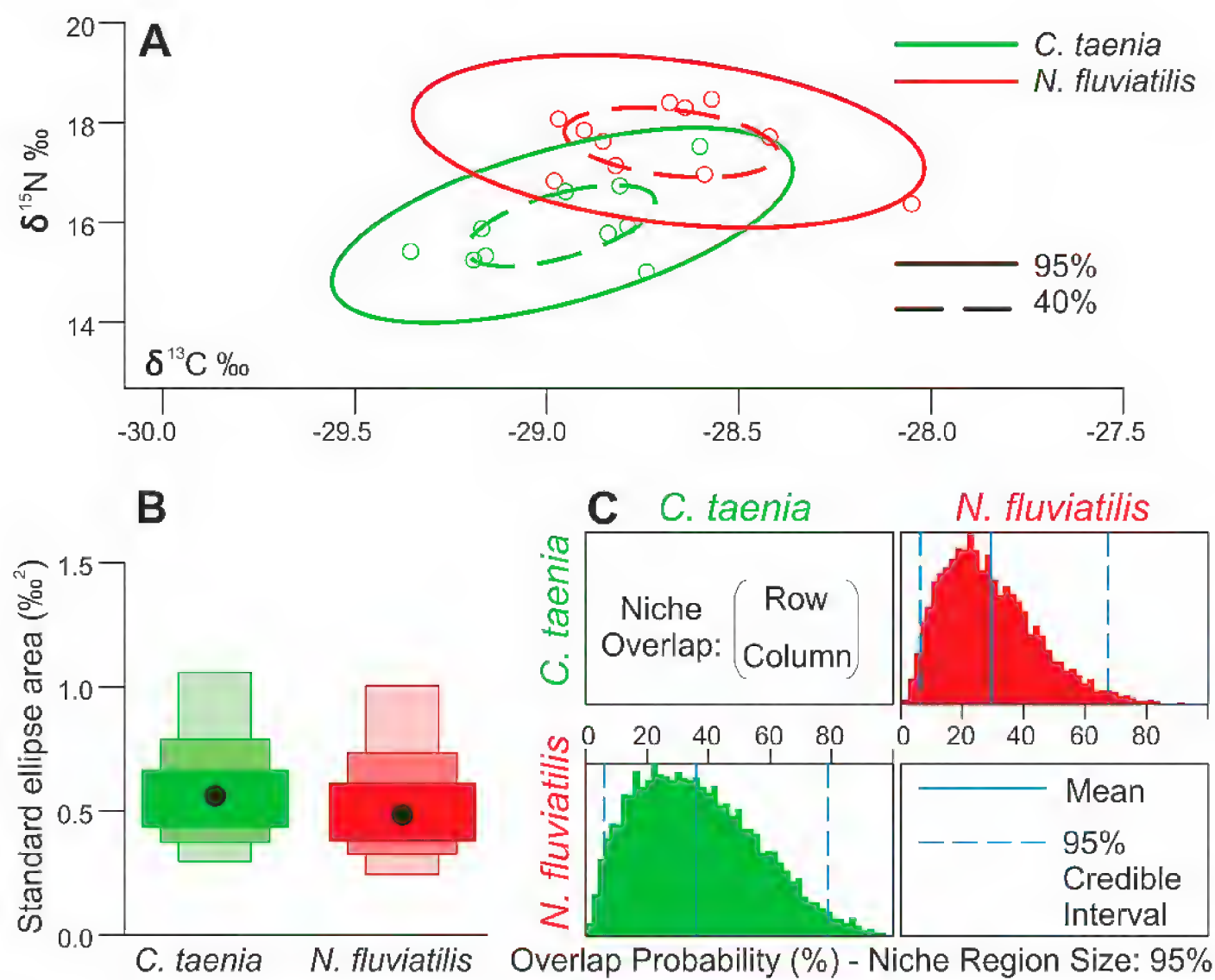
Due to the lack of variability in sand occupation by the spined loach, we ran models testing this variable only for the monkey goby data. The introduction of an intruder, irrespective of its species, increased the time spent by the monkey goby in the sandy substratum (Fig. 5A, Table 4A). Goby intruders exposed to spined loach residents at night spent more time in the sandy substratum than in daylight and in the presence of conspecific residents (Fig. 5B) as shown by a significant resident species\*time interaction (Table 4B). In single species pairs, resident gobies spent more time in the sandy substratum than intruder individuals (Table 4C, Fig. 5C).

The spined loach were more active in the presence of conspecific intruders than in the presence of gobies (Suppl. material 1: fig. S1B, table S5A), as well as at night than in daylight (Suppl. material 1: fig. S1C). Moreover, in daylight, they reduced their activity when an intruder was introduced to the tank, as shown by a

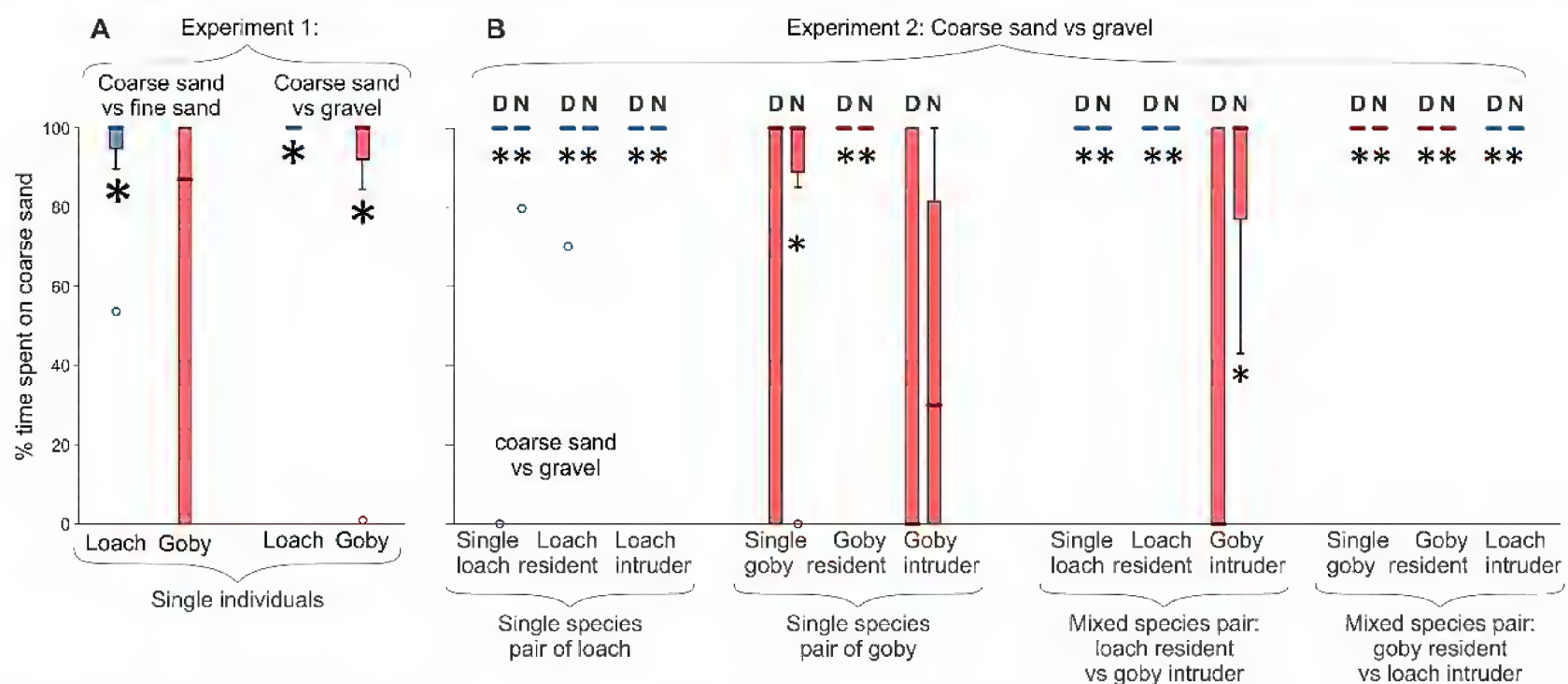
**Table 3.** Substratum preferences of single spined loach (*Cobitis taenia*) and monkey goby (*Neogobius fluviatilis*) in Experiment 1. One sample Wilcoxon tests comparing percentages of time spent by the fish in coarse sand to a theoretical value of 50% (assuming no selectivity).

| Species      | Substrata              | Z     | P      |
|--------------|------------------------|-------|--------|
| Spined loach | Fine vs coarse sand    | -2.46 | 0.014* |
| Spined loach | Coarse sand vs granule | -3.16 | 0.002* |
| Monkey goby  | Fine vs coarse sand    | -0.05 | 0.958  |
| Spined loach | Coarse sand vs granule | -2.27 | 0.023* |





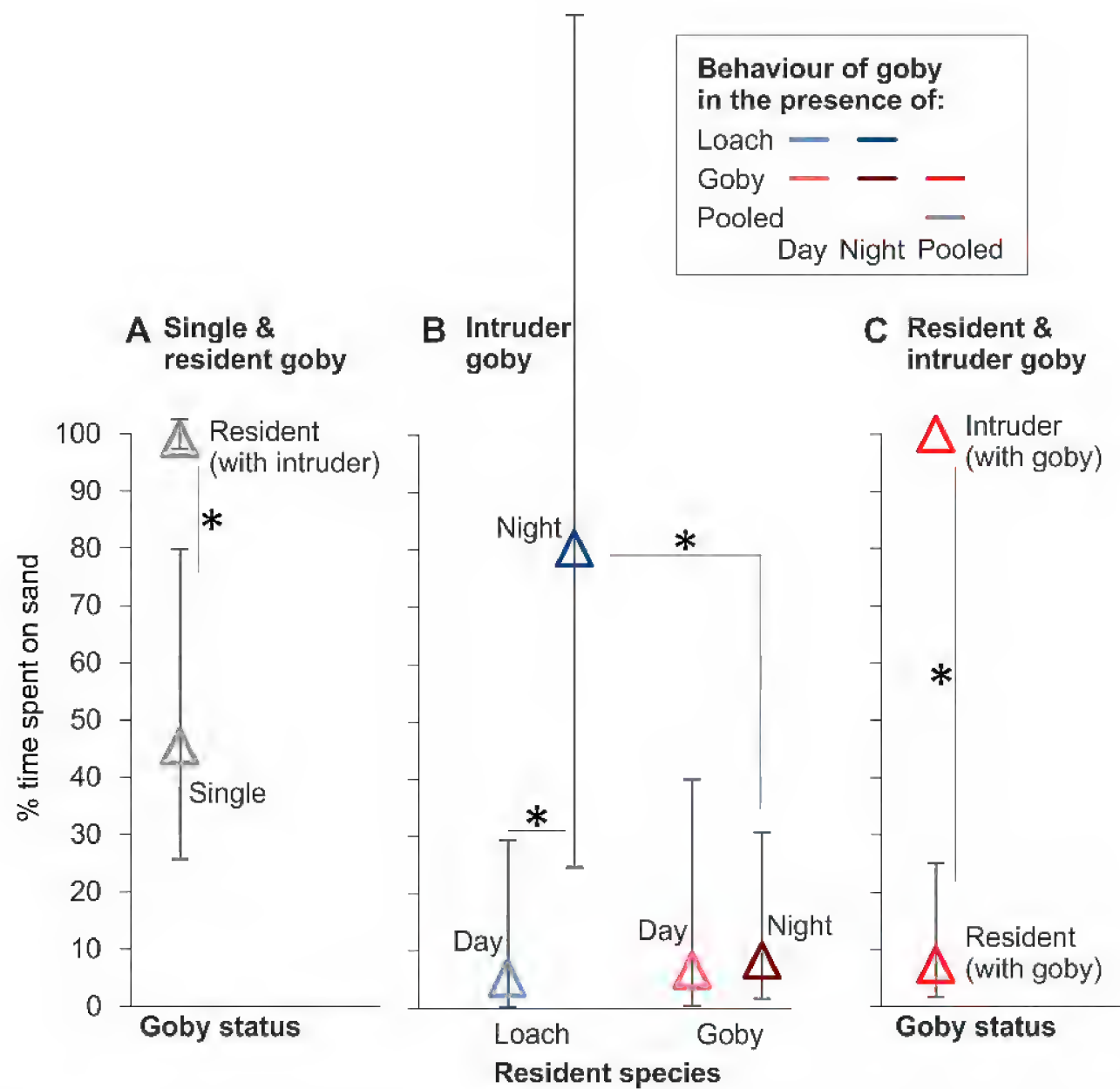
**Figure 3.** A Standard Ellipse Areas (SEA) for spined loach (*C. taenia*) and monkey goby (*N. fluviatilis*): 95% (solid lines) and 40% (dashed lines) B A posteriori distributions for the Bayesian standard ellipse areas (SEA<sub>b</sub>) C Niche overlap based on the 95% confidence interval (SEA<sub>b</sub>).



**Figure 4.** Substratum preferences (percentage of time spent by fish on coarse sand in the presence of alternative substratum) of spined loach (*C. taenia*) and monkey goby (*N. fluviatilis*) A Preference of single fish exposed for coarse sand vs fine sand or granule in Experiment 1 B Preferences of fish in single-species and mixed species pairs in Experiment 2, depending on their status (single fish, residents in the presence of intruders, intruders in the presence of residents). Values differing significantly from 50% (indicated by asterisks, see Table 3 and Suppl. material 1: table S5 for panel A and B, respectively) indicate preferences for coarse sand. Horizontal lines, boxes, whiskers, and circles indicate medians, 1<sup>st</sup> and 3<sup>rd</sup> quartiles, 1.5\* interquartile ranges and outliers, respectively.

significant intruder presence\*time interaction (Suppl. material 1: table S5A). The monkey goby reduced their activity when an intruder was introduced to the tank and were more active in the presence of spined loach vs conspecific intruders, as well as at night vs in daylight (Suppl. material 1: table S5B, fig. S1E–G). The activity of intruder fish of both species was not affected by the resident species. They were more active at night than in daylight (Suppl. material 1: table S5C, D).





**Figure 5.** Time spent on coarse sand by monkey goby (*Neogobius fluviatilis*) in Experiment 2 in single-species and mixed species pairs, depending on their status (single fish, residents in the presence of intruders, intruders in the presence of residents) and time of the day (presentation of significant effects from the models reported in Table 4) **A** Behavior of gobies tested as single fish vs. behavior of goby residents after the introduction of intruders **B** Behavior of goby intruders tested in different times of the day in the presence of various resident species **C** Behavior of residents vs. intruders in single-species pairs. Data are presented as means predicted by the models  $\pm$  95% CI. Asterisks indicate significant differences. The spined loach always spent 100% of time on coarse sand, irrespective of their status, accompanying species, and time of the day.

**Table 4.** Substratum selection (percentage of time spent in sand) by monkey goby (*Neogobius fluviatilis*) in single and mixed species pair in Experiment 2. General Linear Models to test **A** Effect of intruder presence and species on behavior of single/resident individuals **B** Effect of resident species on behavior of intruders **C** Differences in behavior between residents and intruders in single-species pairs. The models additionally included time (day vs night) and relative size (resident/intruder length ratio) to control for their effects. Non-significant higher order interactions dropped from the model in a simplification procedure.

|   | Comparison   | Effect                          | F     | df    | P       |
|---|--|---------------------------------|-------|-------|---------|
| A | Single vs resident fish (with different intruders) | Intruder presence <sup>WS</sup> | 7.64  | 1, 70 | 0.007*  |
|   |  | Intruder species                | 0.00  | 1, 70 | 0.997   |
|   |  | Time <sup>WS</sup>              | 3.40  | 1, 70 | 0.069   |
|   |  | Relative size                   | 0.00  | 1, 70 | 0.999   |
| B | Intruders with different residents                 | Resident species (RS)           | 1.45  | 1, 31 | 0.238   |
|   |  | Time (T) <sup>WS</sup>          | 5.44  | 1, 31 | 0.026*  |
|   |  | RS*T                            | 4.59  | 1, 31 | 0.040*  |
|   |  | Relative size                   | 0.01  | 1, 31 | 0.923   |
| C | Resident vs intruder within each pair              | Individual status <sup>WS</sup> | 19.77 | 1, 33 | <0.001* |
|   |  | Time <sup>WS</sup>              | 0.00  | 1, 33 | 1.000   |
|   |  | Relative size                   | 0.00  | 1, 33 | 1.000   |

<sup>WS</sup> – within-subject factor (repeated measures for the same fish of pair of fish).



Spined loach intruders were more active than residents in daylight, but not at night (Suppl. material 1: fig. S1D), as shown by a significant individual status\*-time interaction (Suppl. material 1: table S5E). The goby intruders were always more active than residents (Suppl. material 1: fig. S1H, table S5F).

## Discussion

Our current study showed that both species, the monkey goby and spined loach, occupied the same habitats at the site of their co-occurrence, their diet composition did not considerably overlap and clearly differentiated isotopic niches also indicated lack of strong food competition. The lack of negative impact of monkey goby on spined loach was additionally demonstrated in experimental condition under the limited resource choice as both species co-occupying preferred substrates and monkey goby did not appear to be stronger competitor than conspecific intruders.

Under natural conditions, both species occupied substrates dominated by coarse and medium sand fractions (1–0.5 and 0.5–0.25 mm, respectively). Most studies on the monkey goby habitat preferences have demonstrated its affinity for sand (Grabowska et al. 2023); however, there are no studies evaluating preferences towards specific substrate fractions (grain diameter), either under natural or experimental conditions. The high association of monkey goby with sand substrate is correlated with its morphology (relatively small head and ventral lobe), leading to strong specialization towards burrowing into soft substrate (Čáková et al. 2008; Grabowska et al. 2023). This habit is believed to help in avoiding predators (Holčík et al. 2003), however, it is also suggested that their strong preference for sandy substrates could limit the expansion of their invasive range (Čáková et al. 2008; Piria et al. 2016). Nevertheless, a significant proportion of sand is associated with high abundances of the species and can be used as a key determinant for its presence (Płachocki et al. 2020; Grabowska et al. 2023). Association of the spined loach with fine-grain substrate has been confirmed in various studies, as well as by its burrowing behavior (e.g. Robotham 1978; Slavík et al. 2000; Pietraszewski 2015). Detailed studies on the spined loach habitat preferences were conducted by Pietraszewski (2015) at the sampling site located in the same river close to our sampling location, but without monkey goby. The species occupied microhabitats dominated by fine and very fine sand and silt (0.250–0.125, 0.125–0.063, < 0.063 mm, respectively) with reduced presence of very coarse sand and granules (1–2 and 2–4 mm, respectively). These results were not confirmed in our study, in which fine and very fine sand and silt constituted less than 2.5% of the substrate, while very coarse sand and granules made up 27% (combined). Among all evaluated points, 39% (37 out of 95) were occupied by both monkey goby and spined loach and there was no significant effect of monkey goby presence on spined loach, regardless of fish size, which suggests the lack of intensive competitive interactions for microhabitats. Čáková et al. (2008) suggested that among the round, bighead, and monkey goby, the last species would have the least impact on native communities. In the Sava River, the presence of monkey gobies initially indicated a decrease in the proportion of gudgeon *Gobio gobio*, suggesting a potential adverse impact (Jakovlić et al. 2015). However, subsequent studies conducted in the same river and similar locations did not confirm these findings (Piria et al. 2016).

In addition to our field results, we carried out laboratory experiments to explore species interactions, specifically examining their habitat selection behaviors. Trials conducted individually showed that spined loach preferred coarse sand over finer and coarser materials, which complements our field observations.



The monkey goby displayed a higher flexibility, not discriminating between fine and coarse sand, while both species avoided granule substrates. In competition trials, there was no observed effect of the monkey goby on spined loach. Both species continued to avoid granule substrates and co-occupied the limited coarse sand patches, indicating no direct competitive interactions between them.

We observed a reduced activity of resident fish compared to their behavior as single individuals immediately after putting them in the experimental tanks. Moreover, intruder fish were more active than resident individuals. This points to the interpretation of increased activity as a response to an environmental stressor (here: a new locality and the presence of another, already established individual). In the case of the spined loach, this hypothesis is also supported by the fact that the above-mentioned differences were exhibited in daylight, where potential environmental dangers are stronger and increased exploration is not likely to be beneficial. Therefore, the higher activity of resident fish (of both species) in the presence of intruding spined loach vs monkey goby, irrespective of the time of the day, suggests that the former intruder poses a stronger negative effect on the behavior of both conspecifics than monkey goby. This is likely to result from the higher general activity of the spined loach in our study. Moreover, only intruding monkey goby moved from their normally preferred sandy substratum to avoided gravel in the presence of conspecifics and spined loach (only in daylight) residents, whereas the substratum selection by the spined loach remained unaffected. Therefore, these results suggest that the presumably invasive monkey goby does not exert a strong effect on coexisting spined loach, affecting their behavior to a lower extent than the presence of conspecific loach. Previous studies suggested no diel pattern in monkey goby activity (Grabowska et al. 2009). Spined loach is usually considered to be more active during the night, but this has been questioned and remains unconfirmed by certain studies (Jażdżewski 2020). Previous laboratory studies have also found the monkey goby to be a benign interference competitor (Van Kessel et al. 2011). Błońska et al. (2016) noted additionally that the influence of the monkey goby on the counterpart species was comparable to that of another conspecific.

Results of our study at the site of both species' co-occurrence confirmed their broad diet spectrum. Monkey goby consumed mostly Ephemeroptera, Chironomidae, Trichoptera larvae and *Asellus aquaticus*, while Chironomidae, Trichoptera and Simuliidae larvae dominated the diet of the spined loach. However, although some prey categories were found in the guts of both species, their relative importance in the diet was different and no distinct diet overlap was observed. The dietary habits of the monkey goby and the spined loach reveal the lack of specialization in prey selection (Jażdżewski 2020; Grabowska et al. 2023).

The trophic ecology of both species indicated a higher trophic position for the monkey goby compared to spined loach, with prey item overlap of only 17%. This finding supports the results of the stomach content analysis. A flexible, opportunistic feeding strategy with a variety of prey items included seems to allow the co-occurrence of monkey goby and spined loach without negative effects.

## Conclusion

When considering non-native species, the most common expectation is their negative impact on recipient communities, which has been supported by numerous studies (Cucherousset and Olden 2011; Britton 2023). However, not all non-na-



tive species pose a similar threat. The research often focuses on cases where abundant invaders lead to significant ecological changes, potentially overstating the impact of non-native species in general (Gozlan 2008; Jackson et al. 2015). Therefore, it is crucial for prioritizing proper management actions (Britton et al. 2023) to report also cases where the impact of non-native species is minimal or relatively benign (e.g., Fobert et al. 2011) or where biotic resistance of native species and ecosystems is considerably high. While analyzing ecological interactions, it is important to consider that spurious correlations can sometimes arise, especially when external factors, such as human impact, simultaneously facilitate invasive species and adversely affect native populations (Tarkan et al. 2012). This may lead to the mistaken assumption that the invasive species directly harms the natives, when in fact, both may be responding independently to the same external pressures. Our comprehensive study showed that, although the monkey goby displays similar diet, habitat, and behavioral patterns as the spined loach, no adverse effect of this non-native species was observed at the studied location. It is possible that habitat partitioning, rather than competition, occurs in this species set, as it has been also observed in other cases involving non-native species (Gjelland et al. 2007; Guo et al. 2012; Kakareko et al. 2016; Grabowska et al. 2024). Despite the monkey goby's increased abundance and extended range, the spined loach population persists (own observ.). Our results do not contradict the invasive character of the monkey goby at other locations and/or in other ecological contexts, e.g. considering other native species or other biological traits. Moreover, the dynamic climate change severely affecting freshwater environments may exert a stronger competitive effect on the spined loach in the future (Fobert et al. 2011). This emphasizes and highlights the importance of context- and site-dependent factors in biological invasions, as the outcomes of interactions between native and non-native species can vary significantly, depending on local environmental conditions and species-specific traits. Such variability highlights and underscores the critical need for site-specific studies to enhance our understanding, prediction, and management of the impacts of invasive species (Błońska et al. 2024).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

DB – conceptualization, data curation, investigation, project administration, writing – original draft; KP, JL – data curation, investigation, formal analysis, visualization, writing – review & editing; BJ – data curation, investigation, visualization; JK – formal analysis, visualization, writing – review & editing; JG – writing – review & editing; AST – formal analysis, visualization, writing – original draft.

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## Data availability

Raw data used in the manuscript that are not already provided are available from the corresponding author on reasonable request.

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## Supplementary material 1

### Habitat and diet interactions in a lowland temperate river suggests no direct impact of non-native monkey goby (*Neogobius fluviatilis*) on native spined loach (*Cobitis taenia*)

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Data type: docx

Explanation note: Table showing diet composition, and a table and figure on preferences and activity from the lab experiment.

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